

## REMARKS

The Examiner rejected claims 1-3, and 10-13 under 35 U.S.C. §103(a) as allegedly obvious over U.S. Patent No. 5,747,246 filed on May 14, 1992 to Pannetier et al. ("Pannetier"), in view of the previously cited Amexis and Ross. Specifically, the Examiner contended that that Pannetier discloses quantifying the absolute amount of at least two target nucleic acid sequences corresponding to at least two genes in a biological sample (see, e.g., the steps set forth in the abstract of Pannetier).

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

Like the previously cited Becker, also Pannetier only teaches using single nucleotide differences if they result in difference in restriction enzyme recognition site (e.g., col. 7, lines 61-65). However, in an Amendment filed on February 26, 2009, Applicants specifically explained, to the Examiner's satisfaction, why one of ordinary skill in the art would not have been motivated to combine references teaching relying on a single nucleotide difference with a methods of Amexis or Ross, which discuss mass spectrometric analysis, and even if one would have combined them, why a skilled artisan would not have expected such a combination to work. A summary of the reasons is provided below.

Applicants submitted a Declaration by Dr. Cantor with the Amendment dated February 26, 2009 ("Cantor Declaration"). Applicants respectfully request the Examiner to review the statements made in the Cantor Declaration, particularly with respect to why one skilled in the art would not have been motivated to use mass spectrometric analysis as claimed in differentiating nucleic acids that only differ by a single nucleotide, particularly for absolute quantification purposes.

While the previously presented arguments address the combination of Becker with Amexis and Ross, they apply similarly to Pannetier, because nothing in Pannetier provides the motivation or changes the expectation of lack of success created by Amexis, Ross and the facts known to skilled artisan.

Specifically, as stated in par. 14 of the Cantor Declaration, Amexis quantified the relative levels of two virus variants in one reaction through PCR and MassArray system. See Amexis, e.g., page 12100, first column, last paragraph. Comparing the relative amount of allelic variants does not allow absolute quantification of nucleic acid species in the reaction. Additionally, because Amexis evaluated relative amounts of allelic products already in the sample, Amexis did not add an external standard.

In par. 15, Dr. Cantor continued that Ross also quantified the relative levels of pooled allelic variants and therefore, for the same reason as Amexis, does not describe how absolute quantification could be achieved. See Ross, e.g., page 624, first column, paragraphs 1 and 2. Also Ross did not use an external standard.

Both Amexis and Ross compared the relative amount of allelic variants; therefore, targets analyzed by the methods described by Amexis and Ross are limited to those targets that comprise an allele (e.g., polymorphism). In contrast, the methods we describe are polymorphism-independent, thus allowing for the absolute quantification of a wider range of targets (e.g., gene sequences that do not contain a polymorphism). (Par. 16 of the Cantor Declaration).

It is well known and also stated in Ross that single base extension, like the one used in the presently claimed methods, produces mass differences between 9 and 40 Da. (Par. 17 of the Cantor Declaration).

However, Ross specifically states that “baseline resolution between alleles differing by 16 Daltons (Da) or less may not be observed” (p. 622, 1st col.). (Par. 18 of the Cantor Declaration).

Ross also states that “area measurement of a low-intensity extension produces within 40 Da of another allele may be confounded by trace cation...adducts onto the lower mass allele” (p. 622, 1st col.). (Par. 19 of the Cantor Declaration).

Therefore, Ross teaches that they made sure that all primer extensions resulted in mass differences between 300-400 Da. Page 622, 1st col.). Ross specifically taught that “two related strategies were selected by which a molecular weight separation of about 300-400 Da between allele products of a given locus could be achieved during the primer extension assay.” See Ross, page 622, paragraph 3, lines 1-6. Ross expected a clear separation of 300-400 Da between alleles and extension products for reliable peak detection and reliable quantification of nucleic acids. One strategy of Ross terminated the variants of the nucleic acid by one (wild-type) and two (mutant) bases, thus enhanced the mass difference; and the other strategy terminated the variants of the nucleic acid by one base (wild-type) and a fluorescently labeled base (mutant). Neither one of the modified primer extension strategies of Ross, is the same as the single-base primer extension method of the present invention. (Par. 20 of the Cantor Declaration).

Single base extension like the one we used, does not produce mass differences of 300-400 Da. (Par. 21 of the Cantor Declaration).

While Pennetier may have described use of a standard for quantification of the amount of the nucleic acid in the sample by using a standard that differs by one nucleotide only, there is nothing in Pennetier that would allow one to expect that such a difference would work using MALDI-TOF.

Therefore, Ross teaches against or away from the method we found to be most effective for absolute quantification purposes. (Par. 22 of the Cantor Declaration).

In view of the above, the base argument and facts remain the same with respect to Pennetier as they were with respect to Ross, namely that one of ordinary skill in the art would not have expected that combination of the mutation analysis of Becker with MALDI-TOF analysis using a single base extension

could be used to provide accurate quantitative measurements of the absolute amount of nucleic acids in a sample (Par. 23 of the Cantor Declaration). Similarly, one of ordinary skill in the art would not have expected that combination of the mutation analysis of Penner et al. with MALDI-TOF analysis using a single base extension could be used to provide accurate quantitative measurements of the absolute amount of nucleic acids in a sample.

In contrast, as already presented in the previous response, we surprisingly discovered that we can accurately quantify the absolute amount of multiple target sequences with multiple internal standards in the same reaction (e.g., triplex targets). We found that the extension products were clearly separated in the mass spectrum with very strong signal to noise level. In particular, the mass differences between several extension products were very small. For example, mass difference between glut3 S and glut3-A was only about 20-25 Da, yet, contrary to what Ross described, we found that the two peaks were clearly separated with strong peak intensities. See September 10, 2007 Response, page 6, last paragraph to page 7, paragraph 2 and Exhibit A. These absolute quantification results by multiplex reactions agreed well with those from uniplex reactions. Moreover, we found that the same method can be used to quantify at least about 20 targets in one multiplex reaction. (Par. 26 of the Cantor Declaration).

In summary, at the time of the invention, absolute quantification of multiple nucleic acids using mass spectrometric detection and single base extension reactions in the same reaction was not something scientists performed or would have expected to succeed. One skilled in the art would not have been motivated to use internal standards with multiplex target nucleic acids for absolute quantification of multiplex without diluting the amplified mixtures, and one would not have been motivated to subsequently use mass spectrometric analysis combined with single-base primer extension for absolute quantification of multiple nucleic acids in the same reaction, particularly when the multiple nucleic acids differentiating only by small mass differences. (Par. 27 of the Cantor Declaration).

Moreover, like stated in par. 12 of the Cantor Declaration:

“if it had been obvious to use Becker to design an absolute quantification method using mass spectrometry, which has been generally known as an analysis tool since at least the mid 1980’s with commercial instruments introduced in the early 1990s, it would not have taken over 10 years from the publication of Becker to develop such a method.

Penner et al. was filed on May 14, 1992 and issued on May 5, 1998. Thus, the above argument by Dr. Cantor applies equally well to Penner et al.

While Penner et al. claims a multiplex reaction of up to 50 targets with 50 standards, measuring such a multiplex reaction using a MALDI-TOF would not have been expected to result in accurate results.

Accordingly, in view of the arguments presented above, Applicants respectfully submit that the rejection of claims 1-3, and 10-13 under 35 U.S.C. §103(a) as allegedly obvious over Pannetier in view of the previously cited Amexis and Ross should be withdrawn.

Applicants submit herewith a Petition and Fees for 3 month extension of time. Applicants believe that no other fees are currently due. In the event that any additional fees are required by this submission, the Commissioner is hereby authorized to charge Nixon Peabody LLP deposit account No. 50-0850 all fees except fees due after allowance.

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Respectfully submitted,

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